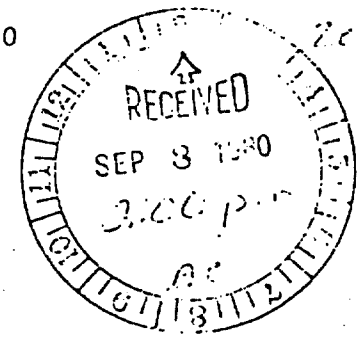
8E1164-0700-00360
86-8000116

(A)

222 RAINBOW BOULEVARD NORTH, BOX 728, NIAGARA FALLS, NEW YORK 14302 PHONE (716) 278-7000

September 4, 1980



Document Control Office
Chemical Information Division
Office of Toxic Substances (WH-557)
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

RE: TSCA §8(e) Submission of Information on the Possible Carcinogenicity of Benzotrichloride, p-Chlorobenzotrichloride and Benzoyl Chloride

Dear Sir:

Attached are four Japanese articles and their English translations that describe toxicity studies on three compounds: benzotrichloride (CAS #98-07-7), p-chlorobenzotrichloride (CAS # 5216-25-1), and benzoyl chloride (CAS # 98-88-4). Also attached is a summary of the adverse effects reported, said summary prepared by P. O. Nees, Corporate Toxicologist, Hooker Chemical Company.

This information, although published, is being reported under the requirements of TSCA §8(e) as described in the EPA Statement of Interpretation and Enforcement Policy; Notification of Substantial Risk Section VI (1) since we could not find reference to the articles through the abstract services listed in Section VII(c).

Further toxicological information on the compounds may be obtained from P. O. Nees, DVM, (716) 278-7414. Information on the production and use of the compounds may be obtained from D. J. Boundy, Product Acceptability Manager, Specialty Chemical Division (716) 278-7888.

Yours truly,

HOOKER CHEMICAL COMPANY

R. L. Noble
Vice President
Environmental Affairs

RJS/mrb18E62

Attachments

Bloomer

July 15, 1980

TO: J. Boundy
S. Gelfand

SUBJECT: Japanese Studies on Benzotrichloride, p-Chlorobenzotrichloride and Benzoyl Chloride

The studies which were recently translated from Japanese were of two types, oral (intubation) and inhalation. The one inhalation study was previously reported (Study V in my previous reviews).

Inhalation Studies

Looking first at the inhalation work, both studies expose animals in a chamber, twice a week for 30 minutes per exposure. One study exposed 5 months and one study for 12 months. In both cases, benzoyl chloride and benzotrichloride were tested in similar manner. In all cases, chemical vapors were generated by drawing air through a flask containing a small volume of the test compound.

The study reports indicate exposure levels of benzotrichloride were 6.8 and 1.6 ppm and were even throughout the exposure period. Considering the rather crude gas generating apparatus and failure to mention any flow control apparatus, the levels of chemicals in the chambers must be questioned even though the 1.6 ppm level was supposedly determined by GLC analyses.

The exposure levels definitely caused inflammatory response in the respiratory tract. This may be essential to the development of tumors. To determine if the benzotrichloride is a chemical carcinogen and not simply an irritant, testing should be conducted at sub-irritant levels. Time of exposure could be increased to provide a similar weekly total dose. The infrequent exposure for a short period results in a very low dose per week. One could compare to 1.6 ppm, 30 minutes per day, 2 times a week to an 8 hour day, 5 days a week exposure at 0.04 ppm.

The results are similar in the two studies. The generation by the system at room temperature or at 50°C did not seem to make any difference in tumor incidences although exposures appeared to cause earlier mortality when 50°C was used to generate the vapors. I would suspect level was enough greater to cause the severity of inflammatory response to increase to a critical level. Regardless, there was high incidence of lung tumors. The reports definitely use the term "cancer" or "cancerous" to refer to the total benign and malignant tumors. Since there is no control data for the inhalation studies, one cannot determine what the real increase over background is. The data on benzoyl chloride (assuming this is negative data) shows some level (3 of) of lung tumors in animals that survived to 14 months. This would be a reasonable, possibly low, level background of lung tumors in mice

from my own experience. The only other "control" data we have on mice in Japan are from the oral administration studies described below. The incidence of spontaneous lung tumors in these mice is extremely low.

There is significant mention of the "leukemia" or "lymphoma" response. Again, in many, if not most strains of mice, this is a common spontaneous lesion. Also the pattern of occurrence (early in the study) is typical of the spontaneous lymphomas, possibly viral in origin. To evaluate the lymphoma incidence one needs to know the caging arrangement. Mice are generally caged in groups of 5 or 10 per cage and the lymphomas frequently occurred in one or two cages and apparently spread from one mouse to another until nearly the entire cage of animals had died of leukemia. I would discount the leukemia as spontaneous and not treatment related until there was positive results from a much more thorough and well designed study.

The skin tumors in the inhalation study also appear to be spontaneous or irritation related. Again, there is no way to evaluate without good control data. In the oral feeding data, there does not appear to be any skin effects from benzotrichloride while p-chlorobenzotrichloride shows a positive response in the high level group and no evidence of spontaneous occurrence in the control group.

Oral Intubation

The two studies, one on benzotrichloride and one on p-chlorobenzotrichloride are nearly identical in their procedure and results. In both cases there was a dose response for both stomach and lung cancer. The stomach cancer was again related to evidence of chronic irritation of the stomach epithelium. Again irritation may be an essential element of the carcinogenic response. Although there is a comment that "keratinous proliferation" was observed even in animals that did not develop tumors, it is not clear whether non-tumor bearing groups also showed the "keratinous proliferation" or the statement referred to non-tumor bearing animals in groups where tumors were found in other animals.

The occurrence of lung tumors in animals dosed by intubation is the most definite indication of chemical carcinogenesis unrelated to irritancy. There is no reference to alteration of the epithelium of the respiratory tract although the potential for direct inhalation exposure does exist if the intubation is not conducted carefully. There is no question there is a dose response, there is adequate control data, and group size is adequate for evaluation.

As in the data on lung tumors, the results reported on skin tumors in groups receiving high levels of p-chlorobenzotrichloride indicate a chemical carcinogen response unrelated to irritation. Again there is the potential for skin contamination but it does not seem likely that the contaminant level would be great enough to act as a skin irritant.

Conclusions

The inhalation studies are equivocal on the basis of lack of control animals. There is also some reasonable doubt regarding the dose level

during the 30 minute exposure period. The importance of the irritation cannot be overemphasized. This may be the mechanism for the carcinogenic effect. Although a cancer is a cancer, if irritancy is a key element, there is a definite cutoff or no effect level. Also uneven exposure levels can result in high, short term peaks which cause the irritation so the reported values may be well below the actual effect level applied.

The data which most strongly indicates that BTC and PCBTC are chemical carcinogens is the evidence of tumor development in lungs and skin after oral intubation. This indicates absorbtion and effect at a remote site, not the site of application.

With the data from the new studies we are only slightly more advanced than we were with the sets of data we reviewed previously. We do have additional data to say that PCBTC and BTC are animal carcinogens and the BOC is not. There is little on which to base an evaluation of the risks related to occupational exposure. The inhalation studies have not been designed as the standard subacute or chronic studies for occupational exposure, i.e. 6 hours per day, 5 days per week.

One other weakness at the present time is the focus on one species, namely the mouse. The mouse is sensitive to halogenated hydrocarbons. Whether the mouse is more or less sensitive to the BTC's and BTF's cannot be determined until some testing is conducted on other species. It would be very helpful if some of the inhalation work were conducted on the rat and/or dog.

With the data available I have to conclude that BTC and PCBTC are carcinogens for the mouse by three routes of administration (oral, dermal and inhalation), and under the conditions of the tests conducted. The part that irritation plays in the carcinogenesis can't be defined at this time. A effect/no-effect threshold concentration for chronic inhalation cannot be determined. The no-effectlevel by oral intubation appears to be approximately 1 to 2 ul/kg/day for PCBTC and BTC. This break point in the studies appears to be about the same for both gastric and lung lesions.

The last inhalation study was conducted in 1979. It may be worthwhile contacting the Japanese researchers to see what additional studies have been initiated. Also there are some key questions which could be answered about the present inhalation work such as:

1. What chamber measurements were made and when were they taken?

2. What was the variability during the exposure periods? What were the peak exposure levels?

3. What is the spontaneous tumor incidence (historical) in the mice (same species and source) used in the inhalation study?

4. What is the purity of the test material?

It may also be possible that the Japanese or some other researchers have determined the irritation threshold level for inhalation of BTC. This information would help in evaluating the results.

With the number of unanswered questions it seems reasonable that the producer/user companies could get together and develop a cooperative program to generate some data and hopefully get some answers.

Paul O. Nees
Corporate Toxicologist

PON42Xbp
7/16/80

Hiroyuki Yoshimura, Hiroo Katayama, Kazuo Takemoto and Shukaku Matsus
Experimental studies by benzotrichloride of the lung tumor (2), Proc.
Japan Assoc. of Ind. Health pp. 332-333, 1979.

347

ベンゾトリクロリド暴露による肺腫瘍発生実験 — II

female mice
1.6 ppm x 30 min x
1.6 ppm x 12 M, left 12
15 M

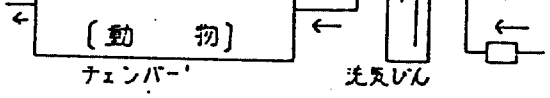
○吉村 博之　　片山 博雄
竹本 和久　　（埼玉医大 公衛）
松下 秀鶴　　（国立公衆衛生院 産衛部）

〔目的〕

塩化ベンジル製造作業者に発生した肺癌等の主因物質である，反応中間体のベンゾトリクロリドの室温吸煙下における暴露実験を行ない，その発癌性を病理組織学的に検討したので報告する。
なお，1977年までに塩化ベンジル製造業者に我が国では5例の肺癌の発生が認められている。（労働省）

〔方法〕

実験動物は，ICR-JCL 雌 マウス 5週令を用い，30分間，週2回，12ヵ月間暴露，以後放置。12ヵ月及び15ヵ月で1群剖剖及び，途中死亡した動物について病理組織学的検索を行なった。暴露方法は，図にあるような方法で，室温（20±5℃）の条件において，600mlの洗気びんに，ベンゾトリクロリド0.5mlを入れ，空気にて気化し，チェンバー内で混和均一化し，暴露を行った。ベンゾトリクロリド濃度は，30分間平均1.6 ppmであった。（濃度測定方法は，FID-GLCによる。）



暴露装置図

〔結果〕

ベンゾトリクロリド室温暴露実験の概要は，表-1にある。実験開始後9ヵ月で死亡したマウスに腫瘍性病変が認められた（線腫）。12ヵ月までの死亡例では気管，大気管支上皮では一部に軽度の腺様化生増殖が認められ（3/4），又，肺には良性線腫（3/4，他の1例は腺様化生増殖）が認められた。11ヵ月死亡例1例には腺癌が発生した。自然病々変は（前回51回本学会発表）50℃条件下での暴露実験では，実験終了（10ヵ月）までに25％（3/12）の発生であったが，本実験では11％（4/37）である。

Month No.　　lung Cancer skin change

表-1　Benzotrichloride : r.t.

期間（月）	例数	上皮増殖		肺腫瘍		皮膚病変	
		気管	肺内気管支	腺腫	癌性	パピローマ	癌 cancer
<12	10(10)*			7	1	1	0
12	10(10)	10	6	5	1	3	1
<15	1(1)			1		0	0
15	9(9)	9	9	2	5	2	5

表-2　Benzotrichloride : r.t.

2~5	12(12)	5	10	2	0	0	0
~12	11(8)	4	9	6	0	0	1
~15	9(1)	0	9	2	1	1	3

表-3　Benzoyl chloride : r.t.

2~5	1(1)	0	0	0	0	0	0
~12	12(7)	0	0	0	0	2	0
~15	1(1)	0	0	1	0	0	0

*（　）内は死亡例数

12ヵ月ト級剖剖例（10例）では，気管，気管支，終末細気管支等の上皮増殖は，ほぼ全例に認められ，1の7例には，一部扁平化生上皮増殖が認められた。肺の腫瘍性変性は，全例に発生し，組織学的に悪性とする腺癌が4例，線腫が5例，腺様化生増殖が1例であった。

15ヵ月ト級剖剖例（9例）では，全例の動物の気管，気管支，終末細気管支等の気道上皮の増殖が認められ，気管上皮の病変では，12ヵ月例より扁平上皮化生増殖が進行し（更年化），一部には上皮の乳化も認められた。又，3例の肺内気管支には乳頭状に発生したpapillomaの発生があった。肺の腫瘍性病変は，ほぼ全例（9/9）に認められ，5例は癌であった。

皮下病変は，8ヵ月頃より発現し，9ヵ月死亡例1例にpapillomaが，12ヵ月ト級剖剖例では1例の複数病巣，3例のpapillomaが認められた。15ヵ月1級剖剖例では，56％（5/9）に皮

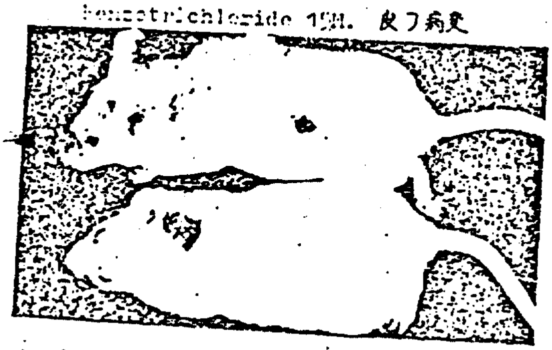
フの腫瘍性病変があり，3例は類表皮癌であった。他臓器の影響では，胃粘膜に軽度の角化増生が認められ，又，多数の動物例において，リンパ・肝臓・脾臓・腎臓等の肥大に伴う炎症性病変が認められた。

なお，表－3に示す様に塩化ベンゾイル暴露（50℃，30分間，週2回，5ヶ月間，以後放置）においても，14ヶ月例の肺に腫瘍性病変が認められ（3/7），うち2例は悪性像を示す腺癌であり，塩化ベンゾイルにも吸入により，肺腫瘍が発生することが認められた。

〔考察〕

以上の実験成績が示す様に，ベンゾトリクロリドは室温条件下（濃度16 ppm）においても吸入により，肺腫瘍（腺腫・腺癌）を発生させる。そして，ほぼ全動物例に，強い慢性気管支炎及び気管支肺炎が発生し，強い気道刺激性が認められる。又，皮フ癌発生も認められ，ベンゾトリクロリドの接触性局所発癌性が証明された。

Benzotrichloride 15M. 皮フ病変

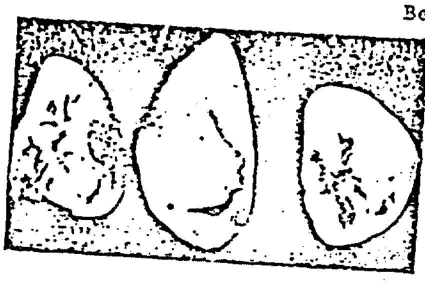


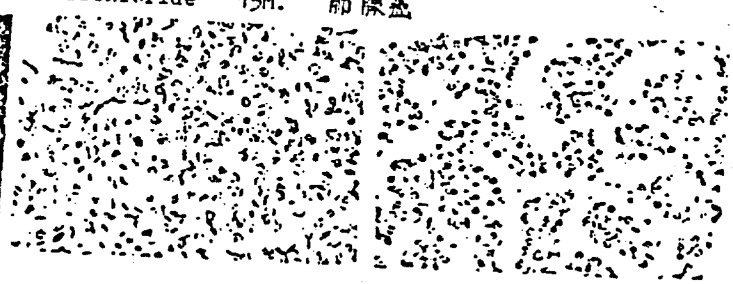
Benzotrichloride 15M. 皮フ類表皮癌

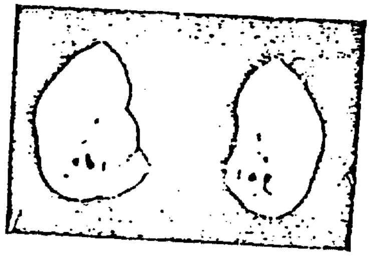
Benzotrichloride 15M. 気管上皮増殖

Benzotrichloride 15M. 気管papilloma

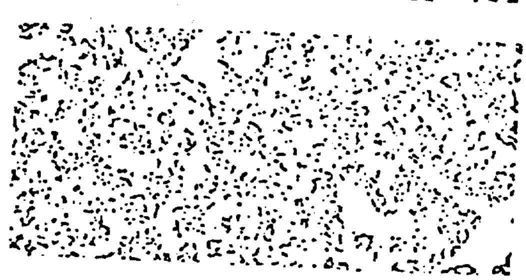
Benzotrichloride 15M. 肺腺癌







Benzoyl chloride 15M. 肺腺癌



TRANSLATION:

EXPERIMENTAL LUNG TUMORS INDUCED BY EXPOSURE TO BENZOTRICHLORIDE

Yoshimura, H., H. Katayama, K. Takemoto* and S. Matsushita**: Benzotorikururorido bakuro ni yoru hai shuyo hassel jikken (II). *Proc. of Japan Assoc. of Ind. Health*, pp. 332-333, 1979.

Purpose of the Study

Experimental animals were exposed at room temperature to benzotrichloride, a reaction intermediate in benzoyl chloride production process and a putative agent of lung cancer among benzoyl chloride workers, and the histopathological features of the animals were evaluated to investigate carcinogenicity of the agent. The results of the study are reported below. It should be added that five cases of lung cancer have been reported among benzoyl chloride workers in Japan up to 1977 (data by the Department of Labor).

Methods

Five-week-old female ICR-JCL mice were used as the experimental animals. The animals were exposed to the agent for 30 minutes twice weekly for 12 months. After the conclusion of the experiment, the animals were reared for an additional 12 or 15 months, at which time they were sacrificed. These animals, together with those which had succumbed earlier, were subjected to autopsy, and histopathological observations were made. They method of exposure is shown in the

*Saitama University Medical School, Department of Public Health.

**National Institute of Public Health, Industrial Medicine Laboratory.

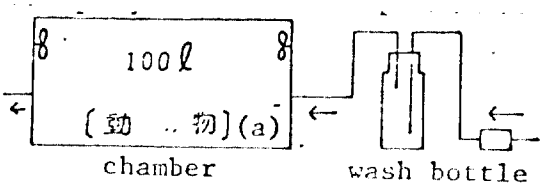


Diagram showing the exposure apparatus.
Key: (a) animal.

diagram above. To illustrate this further, the procedure was conducted at room temperature (20 ± 5°C); and 0.5 ml of benzotrichloride was placed in a 600-ml wash bottle, vaporized in air, and evenly distributed in the air within the exposure chamber. The average concentration in the 30-minute period was 1.6 ppm (the concentration was determined by FID-GLC).

Results

The results of benzotrichloride exposure at room temperature are summarized in Table 1. Cancerous lesions (adenoma) were noted in mice which died 9 months after the start of the experiment. Among the animals which died by 12 months, some had developed mild adenoid proliferation in the tracheal and major bronchial epithelia (3/4), and others were found to have benign adenoma (3/4, one had adenoid proliferation). Adenocarcinomas was found in one animal which died at 11 months. In the test of exposure at 50°C (reported at the 51st Congress of the Association), the incidence of leukemoid lesions was 25% (8/32) at the conclusion of the experiment (10 months), while, in the present test, the incidence amounted to 11% (4/37).

Among the animals sacrificed and autopsied at the end of the 12-month period (10 animals), epithelial proliferation of the trachea, bronchus, and terminal bronchioli was recognized in all, and localized squamous epithelialization in 7. Cancerous lesions were found in all the lungs examined. Among these, malignant

LUNG CANCER SKIN CHANGE

Table 1. Benzotrichloride at room temperature

Duration (months)	Number of animals	Epithelial proliferation		Lung tumor		Skin lesions	
		trachea	pulmonary bronchi	adenoma	malignant	papilloma	cancer
<12	10 (10)*			7	1	1	0
12	10 (0)	10	6	5	4	3	1
<15	8 (8)			3	3	0	0
15	9 (0)	9	9	2	5	2	3

Table 2. Benzotrichloride at 50°C

2 to 5	12 (12)	5	10	2	0	0	0
to 6	11 (4)	8	9	6	0	0	1
to 10	9 (1)	9	9	8	1	4	3

Table 3. Benzoyl chloride at 50°C

2 to 6	8 (1)	0	0	0	0	0	0
to 10	13 (3)	2	0	0	0	2	0
to 14	7 (1)	0	0	1	2	0	0

*( ) indicates the number of animals which died.

adenocarcinoma was found in 4, adenoma in 5, and adenoid proliferation in one.

Among the animals sacrificed at the end of 15 months (9 animals), all were found to have proliferation of the tracheal, bronchial, and terminal bronchiolar epithelia. In comparison with the lesions of the bronchial epithelia of the animals sacrificed and studied at the end of 12 months, the 15-month group were noted to have more advanced squamous epithelial proliferation (stratification),

and some had even developed epithelial keratinzation. Three of these animals had developed papilloma of the pulmonary bronchi. Cancerous lesions of the lung were noted in almost all of these animals (8/9), 5 of which were found to be adenocarcinoma.

Skin lesions began to develop at about the 8th month. Papilloma was found in one animal which died in the 9th month, epidermoid carcinoma in one of those sacrificed at the 12th month, and papilloma in 3, also sacrificed at the 12th month. Among the animals sacrificed at the 15th month, tumorous lesions were found in 56% (5/9). Three of these were epidermoid carcinoma. In the examination of other organs, the gastric mucosa was found to be mildly affected by keratinization. Furthermore, many animals showed inflammatory lesions associated with hypertrophy of organs such as lymph nodes, liver, spleen, and kidney.

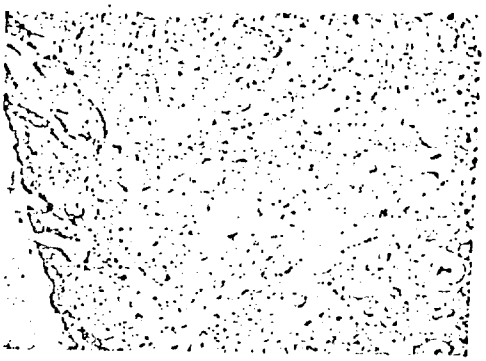
As shown in Table 3, benzoyl chloride exposure (at 50°C, for 30 minutes twice weekly for 5 months, and reared without exposure thereafter) also resulted in tumorous pulmonary lesions in 3/7 of the animals in the 14th month. Among these, two were found to have adenocarcinoma. It was found that benzoyl chloride inhalation could also cause lung cancer.

Discussion

As indicated by the results of the experiment, inhalation of benzotrichloride at room temperature (at a concentration of 1.6 ppm) can cause pulmonary tumors (adenoma and adenocarcinoma). Furthermore, severe chronic bronchitis and bronchial pneumonia developed in all the experimental animals, indicating the strong bronchial irritability of the agent. Skin cancer was also discovered in the experimental animals, which indicated carcinogenicity of benzotrichloride upon local contact.

Benzotrichloride 15M. Skin lesions

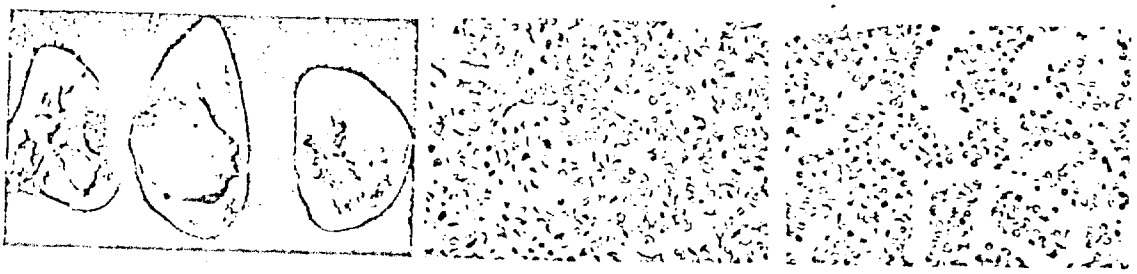
Benzotrichloride 15M.
Epidermoid carcinoma of the skin

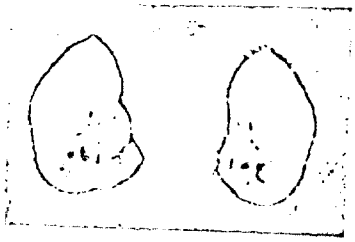


Benzotrichloride 15M. Proliferation of the bronchial epithelium

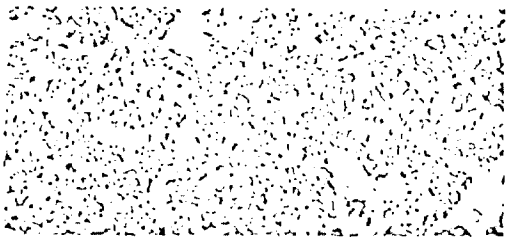
Benzotrichloride 15M.
Bronchus papilloma

Benzotrichloride 15M. Pulmonay adenocarcinoma





Benzoyl chloride 15M. Pulmonary adenocarcinoma



Kazuo Takemoto, Hiroyuki Yoshimura and Shukaku Matsushita: Experiment of pulmonary tumorgenicity of benzotrichloride, Proc. of 51st Annual Meeting of Japan Assoc. of Ind. Health, 1978. (pp. 514-515)

604

ベンゾトリクロリド暴露による肺腫瘍発生実験

○竹本 和夫　吉村 博之 (埼玉医大 公衛)
松下 秀鶴 (産医研)

6.8 ppm x 2/W x 30 min x 5 M
left 1 M or 5 M

本研究の独創性

ベンゾトリクロリドの呼吸器暴露実験による発癌性の証明。マウスを用い，肺・皮膚腫瘍を発生せしめ，ベンゾトリクロリドの強発性局所発癌作用を確認した。

〔目的〕

塩化ベンゾイル製造関連物質に高濃度暴露された作業者に肺癌等の発生が認められ，本学会で松下らが，その主因物質がベンゾトリクロリドであり，又，発癌実験については，マウス塗布実験を行ない報告している。我々は，今回，ベンゾトリクロリドの暴露実験を行ない，その影響を病理組織学的に検討したので報告する。

〔方法〕

実験動物は，ＩＣＲ　マウス，雄，５週令を用い，３０分間，週２回，５ヶ月間暴露を行ない，以後放置。実験開始後６０月及び１０ヶ月で下記剖及び，途中死亡した動物について病理組織学的検索を行なった。　暴露方法は，水浴中（５０±５℃）に固定した６００ ml の洗気びんに，ベンゾトリクロリド 0.5 ml を入れ，蒸発空気にて気化，容量１００ｌのアクリル製チェンバーに導入し，チェンバー内の小型ファンにてガスを混和均一化し，暴露を行なった。

ベンゾトリクロリド暴露濃度は，３０分間平均 6.8 ppm であり，時間・チェンバー内位置で濃度に差はほとんどなく，ほぼ均一であった。

又，ベンゾトリクロリドを沸点（２２０.７℃）まで加温，１回暴露を行なう急性実験を行なった。なお，塩化ベンゾイルについても，ベンゾトリクロリドと同様の方法で暴露を行ない，同様の影響を検討した。

〔結果〕

ベンゾトリクロリド暴露実験結果の概要は表に示す。実験開始後２０月で死亡したマウスの気管，大気管支上皮の一部に中等度の腺様化生増殖が認められ，４０月での死亡例では良性腫瘍が認められた。気管・気管支・終末細気管支等の上皮増殖は，５０月までに死亡した動物の大多数に軽度～中等度の腺様化生増殖が，又，一部の動物には，軽度の扁平上皮化生増殖も認められた。又，５０月までの死亡例では，５０％（6/12）が悪性血病及び白血病であり，５０月以降の死亡・下殺例では，わずかに１０％（3/30）の発生である。動物例はいずれも，脾腫・リンパ・肝臓の肥大が認められ，造血器への侵潤性などから判定したが，実験早期に死亡した動物では，白血病前見による影響が強く表われていると思われる。　６０月下殺解剖例（７例）では，全例に気管・肺内気管支上皮の腺様化生増殖が認められ，その５例には一部扁平上皮化生増殖が認められた。又，肺腫瘍（良性腫瘍）発生は４例であり，そのに，１例には良性に扁平上皮癌の発生が認められた。　１０月下殺解剖例では，全例（8/8）の気管・肺内気管支上皮に腺様化生増殖が，内２例には，肺内気管支に papilloma が認められ，肺腫瘍も全例に認められた。なお，１例は組織学的に悪性度を示す腺癌であり，良性腫瘍も全例多発性のものであった。皮膚の病変は，３例に扁平上皮癌が，４例に papilloma の発生が認められた。造血器への影響は，肝の軽度の脂化増生が数例に認められ，又，大多数の動物で脾腫，炎症性病変を示した。

なお，同時に行なった塩化ベンゾイル暴露群では，全例（２０）に腫瘍発生は認められず，大気・肺内気管支の病変も，１０ヶ月下殺解剖１０例中２例に軽度の腺様化生増殖が認められる程度であり，

発癌性は，著しくベンゾトリクロリドが高く，以下5の皮膚塗布実験結果が示したと同様，明らかに塩化ベンゾイル作業者に発生した肺癌等の主因発癌物は，ベンゾトリクロリドである。

急性暴露の病果は，暴露24時間後では，気管粘膜も脱落・上皮細胞は膨化し，炎症細胞の浸潤が著しく，肺内組織では，好中球を主体とした広範な細胞反応が認められた。　1週間後暴露，小気管支は，線毛・円柱細胞・杯細胞は脱落し，2ないし3層上皮に軽度の異型度を示す細胞が増生していた。

又，2週間後見では，気管上皮は大部分基底細胞のみ残存し，線毛・円柱細胞・杯細胞は脱落している。しかし，一部では3ないし4層が重層化した細胞が認められ，暴露後，約2週間　皮の再生機構があると考えられる。

〔考察〕

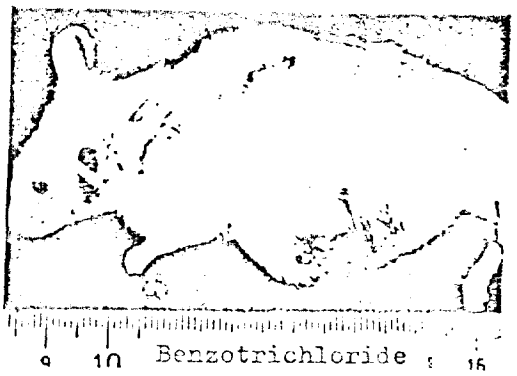
以上り暴露実験り結果，ベンゾトリクロリドは吸入により，気管・気管支り上皮増殖を発生させ，又，肺実質部においても線維を多発させ，一部に悪性化を見している。これ等り結果，ベンゾトリクロリドが気道性に局所発癌性を示すことが明らかであり，又，皮膚癌発生もこり傾向を示すものである。

ベンゾトリクロリド暴露結果

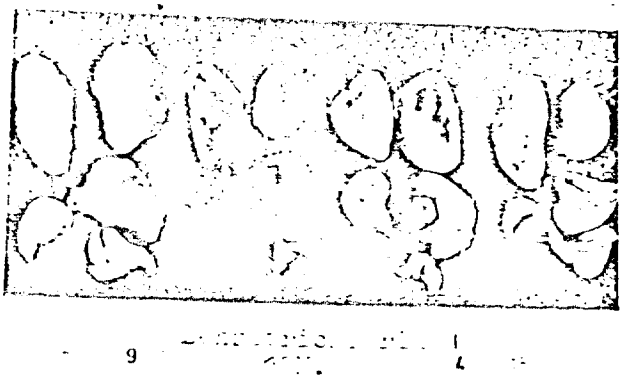
期間(月)	例数	上皮増殖		肺腺腫	皮膚		類白血病及び白血病
		気管	肺内気管支		papi.	癌	
		trachea	lung	lung			
2~4	7(7)※	2	5	1	0	0	6/12
~5	5(5)	3	5	1	0	0	
~6	11(4)	8	9	6	0	1	2/20
~10	9(1)	9	9	9	4	3	

※（　）内は死亡例数

skin　paraleukemia &leukemia



10ヶ月．皮膚癌例．
skin cancer



10ヶ月．　肺腺癌及び腺腫
adenocarcinoma

10ヶ月．気管の扁平上皮癌生　　10ヶ月　乳頭状の肺腺腫
squamatous cell carcinoma　　papilloma

急性実験　上皮再生期
acute experiment

TRANSLATION:

EXPERIMENT OF PULMONARY TUMORGENICITY OF BENZOTRICHLORIDE

Takemoto, K.*, H. Yoshimura* and S. Matsushita**: Benzotorikurorido bakuro ni yoru hai shuyo hassei jikken. *Proc. of 51st Annual Meeting of Japan Assoc. of Ind. Health*, pp. 514-515, 1978.

Originality of the Present Study

Establishment of carciongenictiy of benzotrichloride in respiratory system by localized exposure; pulmonary and skin tumors were induced in mice to confirm the carcinogenic action of benzotrichloride by local contact.

Purpose of the Study

The incidence of lung cancer was recognized among workers who had been exposed to a high concentration of substances related to benzoyl chloride. At the present Congress, Matsushita et al. reported that the main component of these substances is benzotrichloride; its carcinogenicity was proven by their experiment (in which mouse skin was painted with the putative chemical). We have conducted a test on benzotrichloride exposure and observed its histopathological effects. The results are reported in the following.

Methods

Five-week-old female ICR mice were selected as the experimental animals.

*Saitama Medical University, Department of Public Health.

**Industrial Medicine Research Institute.

The animals were exposed to benzotrichloride for 30 minutes twice weekly for 5 months. At the end of this time, the animals were reared without further exposure, sacrificed, and subjected to autopsy 6 and 10 months after the start of the experiment. Histopathological observations were made on these animals together with those which succumbed during the experiment. For exposure, 0.5 ml of benzotrichloride was placed in a 600-ml wash bottle, stabilized in a water bath (50 ± 5°C), vaporized in a dry atmosphere, and conducted into an acrylic chamber with a 100 ℓ content. A small fan was attached to the chamber to distribute the gas evenly within.

The average concentration of benzotrichloride amounted to 6.8 ppm throughout the 30-minute test period. This concentration was found to be uniform throughout the test period regardless of the locations within the chamber.

In an acute toxicity test, benzotrichloride was heated to the boiling point (220.7°C) and the animals were exposed to the gas once. Benzoyl chloride exposure test was also conducted in a similar manner to test its chronic effects.

Results

The results of benzotrichloride exposure are summarized in the table. Moderate adenoid hyperplasia was noted in part of the trachea and major bronchi of mice that expired at two months after the start of the experiment. Development of benign adenoms was found in those which expired in four months. Epithelial proliferation of the trachea, bronchus, and bronchioli was manifested as mild to intermediate adenoid proliferation in the majority of the animals that expired by the end of five months. Mild squamous epithelialization was also noted in some. Among the animals which succumbed by the fifth month, 50% (6/12) were affected by para-leukemia and leukemia, while the incidence of leukemia was only

THE RESULT OF BENZOTRICHLORIDE EXPOSURE.

Period	No.	Epithelial proliferation		Pulmonary tumor	Skin		Paraleukemia and leukemia
		trachea	pulmonary bronchi		papi.	cancer	
2-5	7 (7)*	2	5	1	0	0	6/12
5	5 (5)	3	5	1	0	0	
6	11 (4)	8	9	6	0	1	2/20
10	9 (1)	9	9	9	4	3	

*( ) indicates number of animals which died.

10% (2/20) among those which died after the initial five months or were sacrificed at the end of the experiment. All animals had hypertrophy of the thymus, lymph nodes, and spleen, suggesting invasiveness of the condition to other organs. Among the animals that died early in the experiment, the effects of the leukemic condition were more pronounced. Adenoid proliferation of the tracheal and intra-pulmonary bronchial epithelia was found in all the animals sacrificed at the end of six months (7 animals), and partial squamous cell epithelialization in 5 [out of 7] animals. Lung tumors (benign adenoma) had developed in 4 animals, one of which also had squamous cell carcinoma of the skin. Among the animals sacrificed at the end of ten months, all (8/8) were found to have developed adenoid proliferation of the tracheal and intrapulmonary bronchial epithelia and 2 had developed papilloma of the intra-pulmonary bronchi. Pulmonary tumors were found in all the animals. One was adenocarcinomas with malignant histological features. All benign tumors were in multiple incidences. Among the dermal lesions, 3 indicated development of squamous cell carcinoma and 4 had papilloma. In observations on the involvment of other organs, mild keratocyte proliferation of the stomach

was noted in several. The majority of the spleens showed inflammatory changes.

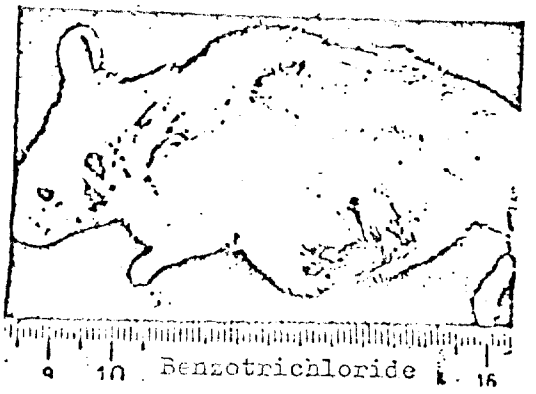
In the benzoyl chloride exposure test conducted simultaneously, all 20 animals were free of lung cancer. The lesions of the trachea and intra-pulmonary bronchi were limited to mild adenoid proliferation in 2 out of 10 animals which were sacrificed at the end of ten months. Evidently, carcinogenicity of benzotrichloride is much more potent. As indicated in the study by Matsushita et al., by coating the skin, the causative agent of lung cancer among the workers exposed to benzoyl chloride was concluded to be benzotrichloride.

In the acute experiment, the bronchi were characterized by loss of cilia, enlargement of the epithelial cells, and marked infilatration of inflammatory cells at the end of 24 hours after exposure. The pulmonary tissues were marked by extensive alveolitis with the dominant presence of eosinophils. After a lapse of one week, the bronchioli were characterized by losses of cilia and columnar and goblet cells and proliferation of cells with a mild atypia in 2 or 3 epithelial layers.

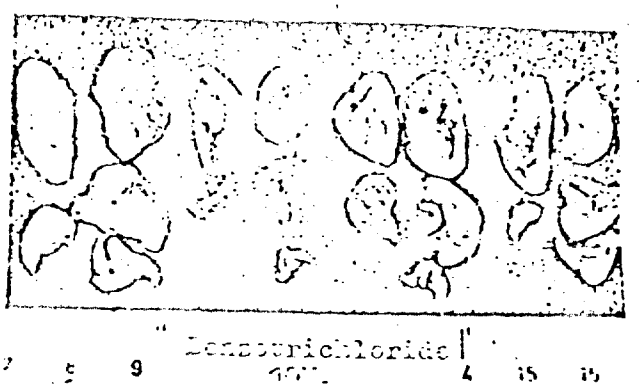
After a lapse of two weeks, cilia and columnar and goblet cells all prolapsed, and most of the bronchial epithelium was marked by the presence of basal cells only. However, some cells appeared in 3 or 4 layers, indicating the possible development of epithelial regeneration within two weeks following exposure.

Discussion

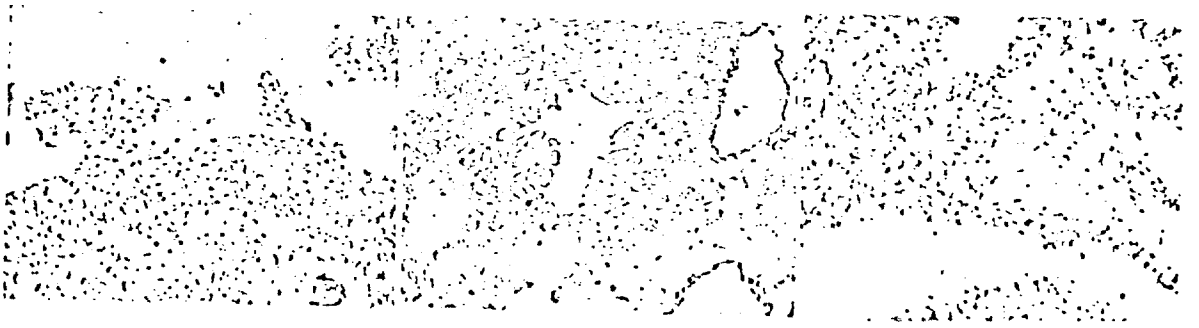
The above test results indicated that inhalation of benzotrichloride causes epithelial proliferation of the trachea and bronchi and multiple development of adenomas in the pulmonary parenchyma with malignant changes in part. It is evident that benzotrichloride has a local carcinogenic effect on the respiratory tract. It was also found to be carcinogenic to the skin.



Skin cancer at 10 months.



Pulmonary adenocarcinoma and adenoma at 10 months.



Squamous epithelialization of the bronchi at 10 months.

Papilloma of the middle bronchus at 10 months.

Acute experiment, swelling of epithelial cells.

Kazuo Fukuda, Shukaku Matsushita and Kazuo Takemoto: Carcinogenecity of orally administered benzotrichloride, Proc. of 51st Annual Meeting of Japan Assoc. of Ind. Health, 1978. (pp. 516-517)

605

ベンゾトリクロリドの経口発癌

female mice 40 each
or 2, 0.5, 0.125, 0.315
ul/head x 2/W x 25W
Left for 18 M
(including
25 W)

○福田一男* 松下秀鶴* 竹本和夫**
(*産医研 **埼玉医大・公伝)

本研究の独創性

塩化ベンジル製造作業者に発生した膀胱癌の主因物質であるベンゾトリクロリドの経口投与により、投与局所の腺胃を含む比較的全身性の腫瘍の発生が認められ、かつ投与量と腫瘍発生数との間には、いわゆるDose-Responseの関係があった。また先に皮膚塗布実験で発生した消化器系腫瘍は当該物質の嚥下によるものと考えられた。

〔目的〕 演者らは塩化ベンジル製造作業者に発生した膀胱癌の主因物質は、ベンゾトリクロリドであることを既に本学会で発表した。そのときベンゾトリクロリドの皮膚塗布実験で認められた消化器系腫瘍は、当該物質の経口摂取によるものと想定されたが、一方投与経路を異にした場合、皮膚塗布実験で投与局所以外に認められた腫瘍の発生分布が変わることが考えられたので、これらを確かめるため、ベンゾトリクロリドの経口投与実験を行なった。

〔方法〕 9週令のICR系雌マウスを1群40頭づつ用い、ベンゾトリクロリドを1回あり各々2.0μl（A群）、0.5μl（B群）、0.125μl（C群）、0.0315μl（D群）を含むゴマ油0.1mlを胃ゾンデで投与した。週2回、25週間、合計50回投与した後、投与開始後18ヶ月で剖検し、病理組織学的検索を行なった。

〔結果〕 実験結果の概要を表1および図1、図2に示す。表では同一臓器での原発腫瘍はその悪性度の強い方を採り、同一個体の重複腫瘍はその種類と全て数に入れてある。死亡率は高濃度群が早く、全動物の50%死亡率はA群が6.5ヶ月、B群が16.4ヶ月で、C群以下は18ヶ月の時点でも50%に達しない。

表および図から判る様に、腫瘍発生時期は高濃度投与のA群が他群に比して著しく早く、以下投与量の多い群の方が早い傾向にある。投与量と腫瘍発生との間には、いわゆるDose-Responseの関係が認められる。最も早期に発生する腫瘍は前胃癌で、投与6ヶ月迄にA群の20%（7/35）、B群の5%（2/40）に認められ、その組織型はリンパ腫のみで、上皮型癌りは混合型はない。最も高率に発生する腫瘍は前胃の扁平上皮がんで、その発生率は12ヶ月迄にA群で67%、18ヶ月迄にB群で55%、C群で5%、D群で0%である（但し、Ca. in situを含まず。図1）。転移はリンパ行性、播種が大部分で、血行性は少ない。又、腫瘍発生が認められなかった個でも角化増生が認められる。これに対して腺胃では、粘膜肥厚、乳頭腺増生、上皮化生増殖等が少数例認められるが、腺がんは認められない。前胃がんに次いで高頻度に発生する腫瘍は、肺腺がんおよび前胃癌であるが、腺扁平上皮がんは認められない。その発生率を図2に示す。本実験では外分泌腺である耳腺、唾液腺、涙腺等の腫瘍が、投与群合計150頭中10頭（7%）に発生し、又、前胃基底細胞癌が1例ではあるが認められた事は興味深い。但しそれら外分泌腺腫瘍発生頻度と投与量との間には関係がない。

〔考察〕 ベンゾトリクロリドの胃内投与により高率に発生する腫瘍は、投与局所の胃がんであり、先に皮膚塗布実験で認められた皮膚がん、胃がんは、動物が塗布部位を舐めたためと考えられる。塗布実験部位に肺がんが発生するか、その発生率は塗布の場合より小さく、又、今回の経口投与実験では耳腺、唾液腺、前庭腺等に腫瘍発生が認められるが、これは塗布実験では起り得ていない。一方、ベンゾトリクロリドのマウス全身曝露での吸入実験で、肺がん、皮膚がん、白血病などで認められている（竹本ら、本学会発表）。これらのことは、ベンゾトリクロリドは強力な皮膚がんのみならず広く全身性の発がん性があるものと考えられ、ベンゾトリクロリドの代謝機構に発癌性に有ることと合せて考えるとその発がん作用は興味あるものであり、その作用は今後の研究にまたれる。

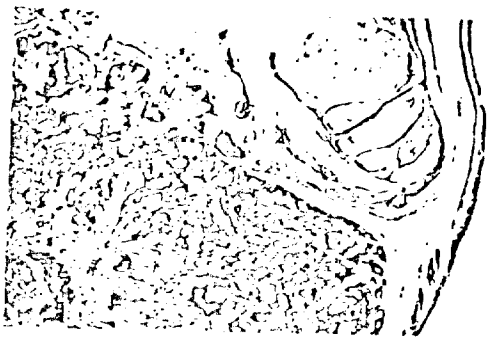


写真1. A群の扁平上皮がん. 160日目死亡
squamatous cell carcinoma of the stomach

写真2. A群の肺腺がん. 192日目死亡
adenocarcinoma of the lung

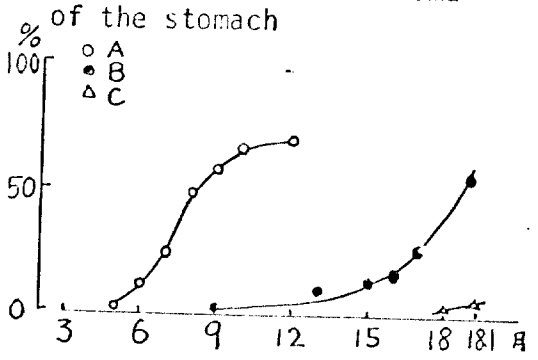


図1. 胃がん, 食道がんの累積発生率
Accumulated rate of stomach and oesophagus cancers

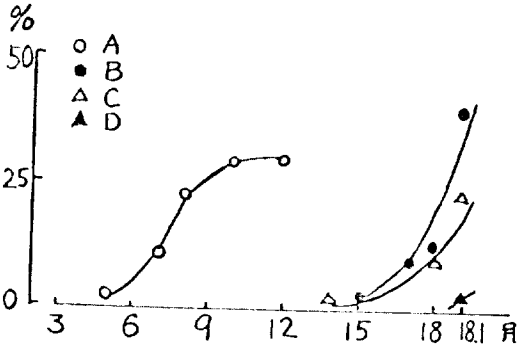


図2. 肺がんの累積発生率
Accumulated rate of lung cancer

TABLE 1. TUMORS INDUCED IN ICR-SLC MICE FOLLOWING ORAL ADMINISTRATION OF BENZOTRICHLORIDE

GROUP	Initial No. of animals	Effective No. of animals	Forestomach			Oesophagus Sq. cell ca.	Lung		Syrinx oma		Salivary Gland		Harderian gland adenocarcinoma	Malignant Lymphoma	Thymoma	Liver Hemangioendothelioma	No. of Animals with tumor(s)		
			Sq. cell ca.	Ca. in situ	Polyp		Adenocarcinoma	Adenoma	Malignant	Benign	Ca.	Ad.					Malignant	Benign	Total
A 2 μl	40	35	24	2	1		10	10	2		1				7		27	6	33/35
B 0.5 μl	40	40	22	3		1	16	17	1		2			1	2		28	8	36/40
C 0.125 μl	40	38	2	2			9	6			1	1			1		11	9	20/38
D 0.0315 μl	40	37		1			1			1			1	1		1	2	3	5/37
Cont.	40	35						1									0	1	1/35

1). Sq. cell ca. : Squamous cell carcinoma. 2). Ca. : Carcinoma.
3). Ad. : Adenoma. 4). Lung adenoma : Multiple adenoma only.
5). Carcinoma in situ in the forestomach was regarded as benign tumor.

TRANSLATION:

CARCINOGENICITY OF ORALLY ADMINISTERED BENZOTRICHLORIDE

Fukuda, K.*, S. Matsushita* and K. Takemoto**: Benzotorikuroridо no keiko hatsugan. *Proc. of 52st Annual Meeting of Japan Assoc. of Ind. Health*, pp. 516-517, 1978.

Originality of the Present Study

Benzotrichloride, a causative agent of malignant tumors among benzoyl chloride workers, was given orally, and development of tumors was noted in almost the entire body as well as the organ coming into direct contact with the test agent. Furthermore, a so-called dose-response relationship was recognized between the dosage and the number of the tumor-bearing animals. It was believed that the gastrointestinal tumors found in the previous test (in which the test agent was painted on the skin surface) were caused when the animals ingested the agent.

Purpose of the Study

The speakers have already reported at the previous meeting that the putative agent in the development of lung cancer among benzoyl chloride workers was benzotrichloride. In the same study, incidences of gastrointestinal cancers were noted in the test in which the test agent was painted on the skin of the animals. It was presumed that the agent was somehow orally taken by the animals. It was projected that, upon changing the administration route, the pattern of distribution of tumors in sites other than the area of contact in the skin coating test

*Industrial Medicine Laboratory.

**Saitama University Medical School, Department of Industrial Health.

may also vary. For this reason, oral administration of benzotrichloride was attempted.

Methods

Nine-week-old female ICR mice were selected as the experimental animals. Forty animals were assigned to each group. 0.1 ml of sesame oil containing 2 µℓ (Group A), 0.5 µℓ (Group B), 0.125 µℓ (Group C), or 0.0315 µℓ (Group D) [of benzotrichloride] was administered to each animal via a stomach tube. The administration was scheduled twice weekly for 25 weeks (total, 50 times). After a lapse of 18 months since the start of administration, the animals were subjected to autopsy, and histopathological observations were made.

Results

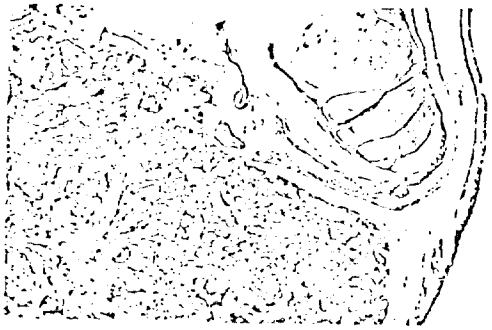
The results of the experiments are summarized in Table 1 and Figures 1 and 2. Among the primary tumors occurring in an organ, those with a higher malignancy were entered in the table. Multiple tumors in a single individual were included individually in the count. The groups with higher dosages had higher mortality in a shorter time. Fifty percent mortality occurred in 6.5 months (Group A) and 16.4 months (Group B). Fifty percent mortality had not been reached by the 18th month in Groups C and D.

As is evident in the table and figures, in Group A, tumors occurred much sooner than in the other groups. In general, tumors developed earlier in the groups which received higher dosages, indicating a so-called dose-response relationship between the dosage and the development of tumors. The tumor that developed earliest was thymoma, which appeared within six months in 20% of Group A animals (7/35) and in 5% of Group B animals (2/40). Its histological type was

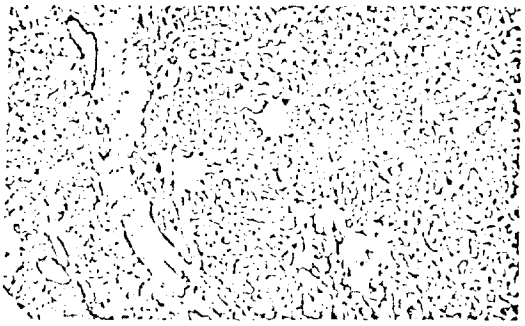
TABLE 1. TUMORS INDUCED IN ICR-SLC MICE FOLLOWING ORAL ADMINISTRATION OF BENZOTRICHLORIDE.

GROUP	Initial No. of animals	Effective No. of animals	Forestomach			Oesophagus	Lung		Syrinx-oma		Salivary Gland		Harderian gland adenocarcinoma	Malignant lymphoma	Thymoma	Lever Hemangioendothelioma	No. of animals with tumor(s)		
			Sq. cell ca.	Ca. in situ	Polyp	Sq. cell ca.	Adenocarcinoma	Adenoma	Malignant	Benign	Ca.	Ad.					Malignant	Benign	Total
A 2 μl	40	35	24	2	1		10	10		2		1			7		27	6	33/35
B 0.5 μl	40	40	22	3		1	16	17	1			2		1	2		28	8	36/40
C 0.125 μl	40	38	2	2			9	6			1	1			1		11	9	20/38
D 0.0315 μl	40	37		1			1			1			1	1		1	2	3	5/37
Cont.	40	35						1									0	1	1/35

1) Sq. cell ca. : Squamous cell carcinoma; 2) Ca. : Carcinoma.
3) Ad. : Adenoma; 4) Lung adenoma : Multiple adenoma only;
5) Carcinoma *in situ* in the forestomach was regarded as benign tumor.



Photograph 1. Squamous cell carcinoma of the stomach of Group A. The animal succumbed on the 160th day.



Photograph 2. Adenocarcinoma of the lung in Group A. The animal succumbed on the 192nd day.

limited to the lymphatic, but epithelial or mixed types were absent. The most frequently seen tumor was squamous cell carcinoma of the forestomach. Its incidence up to 12 months was 67% in Group A; and up to 18 months, 55% in Group B,

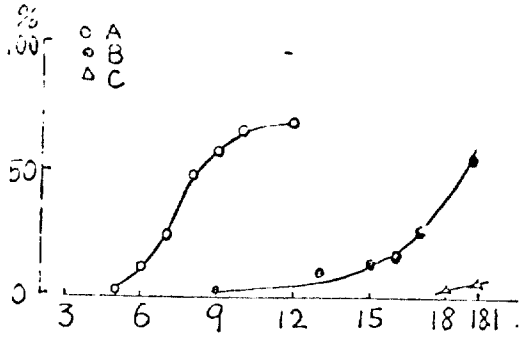


Figure 1. Cumulative incidences of gastric and esophageal cancers.

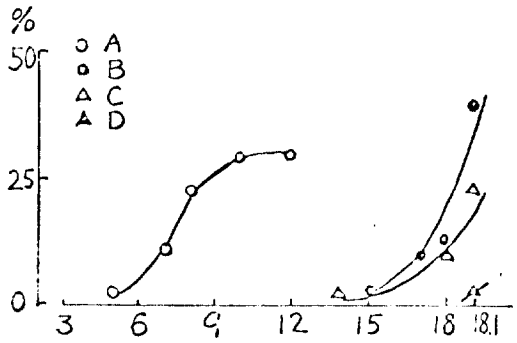


Figure 2. Cumulative incidences of pulmonary cancer.

5% in Group C, and 0% in Group D (the data do not include carcinoma *in situ*; ref. Figure 1). Most of the metastases were of a lymphatic or seeding type, but few were of a hematogenous type. Keratinous proliferation was recognized even in the animals which were free of tumors. On the other hand, in the glandular stomach, hypertrophy of the mucous membrane, atypical adenoid proliferation, and epithelial proliferation were observed in a few, but no development of adenocarcinoma was recognized. The most frequently occurring cancers next to that involving the forestomach were adenocarcinoma and adenoma of the lung. No pulmonary squamous cell carcinoma was discovered. The incidences of these tumors are shown in Figure 2. Tumors involving exocrine glands such as sweat glands, salivary glands, and lacrimal ducts developed in 10 (7%) out of 150 animals treated. Endothelioma of the vessels supplying the liver was found in one animal. These present interesting problems. No relationship was found between the dosages and the incidences of exocrine gland tumors.

Discussion

Intragastric administration of benzotrichloride results in high incidences of cancer of the stomach which comes in direct contact with the

agent. Esophageal and gastric cancer developing in the experiment in which the skin was coated with the test agent were believed to be due to the licking of the coated skin by the animals. As in the skin-coating test, lung cancer was recognized in the present test, and its incidence was higher. The current oral administration also resulted in development of tumors involving the sweat gland, salivary gland, and hepatic vascular endothelium, which were absent in the skin-coating test. In the exposure of mice to benzotrichloride in the inhalation test, lung and skin cancers and leukemia were recognized (Takemoto et al., reported at the present Congress). These findings serve to prove that benzotrichloride acts not only as a local, but also as a systemic carcinogen. Together with the fact that the metabolites of benzotrichloride act as mutagens, its carcinogenic action presents an interesting problem. Future studies on the analysis of this problem are expected.

Kazuo Fukuda, Shukaku Matsushita and Kazuo Takemoto: Carcinogenicity p-chloro-benzotrichloride, Proc. pf Japan Assoc. of Ind. Health pp. 331, 1979.

P－クロロベンゾトリクロリドの発癌性

female mice 30 each; or 2, 0.8, 0.32, 0.13 ul/head x 2/W x 17.5 W left 18 M (including 17.5W)

○福田一男*，松下秀鶴**，竹本和夫***
（*労医研，**労医研・国立公衆衛生院，
***埼玉医大・公衛）

〔目 的〕 塩化ベンゾイル製造工程で取扱われる主原料及び主生成物は，トルエン，ベンゾトリクロリド，塩化ベンゾイル，安息香酸，塩素ガスなどであるが，ベンゾトリクロリドと塩化ベンゾイルに発がん性があることは前回までの本学会に既に発表した。本製造工程での反応副生物又は不純物には，塩化ベンジル，塩化ベンザル，O－及びP－クロロベンゾトリクロリド，O－及びP－クロロ塩化ベンザル等があるが，前四者には強弱の差はあるがいずれも発がん性があることを演者らは認めている。特にP－クロロベンゾトリクロリドはベンゾトリクロリドと同程度の強い発がん性を有していた事が皮膚塗布実験で認められたので（表1），更に経口的に投与した時の腫瘍発生と用量との関係を追求した。

〔方 法〕 8週令のICR－SLC系雌マウスを1群30頭ずつ用い，P－クロロベンゾトリクロリドを1頭当り各々2 μl（A群），0.8 μl（B群），0.32 μl（C群），0.13 μl（D群），0.05 μl（E群）を含むゴマ油0.1 mlを胃ゾンデで投与した。週2回，17.5週間，合計35回投与した後，投与開始後18ヶ月で剖検し，病理組織学的検索を行なった。

〔結 果〕 実験結果の概要を表2及び図1，2，3に示す。表2では同一臓器での[illegible]はその悪性度の高い方を採り，同一個体の重複腫瘍はその種類を全て数に入れてある。又，[illegible]置後平均月令は18ヶ月での屠殺動物も含む。全動物の50%死亡率は高濃度投与のA群が4.7ヶ月と著しく早く，次いでB群が12.3ヶ月で，C群以下は18ヶ月の時点でも50%に達しない。[illegible]時期は高濃度投与のA群が他群に比して早い。最も早期に発生する腫瘍は悪性リンパ腫及び胸腺腫で，その発生率は投与開始後7ヶ月迄にA群38%（11/29），B群10%（3/29）である（図1，表2）。投与局所の胃がんは，A群では投与開始後5ヶ月ごろから発生するが，その累積発生率はいずれの群も25%以下である（但し，Ca. in situ を含まず，図2）。E群に1例の分化型腺管状腺がんを認める以外，全て扁平上皮がんである。前胃の上皮内がんがC群以上の高濃度群に認められるが，多発性乳頭腫は投与全群に発生する（表2）。前胃上皮の角化増生は高濃度群ほど著明であり，[illegible]上皮が[illegible]濃度群に認められる。腺胃では上皮の[illegible]，[illegible]，[illegible]化生等が[illegible]在する。[illegible]も高率に発生する腫瘍は肺腫瘍であるが，その[illegible]は腺がん，腺腫のみで其他の腫瘍は認められない。多発性腺がん等で[illegible]にある[illegible]がんなども存在する。18ヶ月迄の発生率は[illegible]高濃度群の方が高い（図3）。又，皮膚がん（扁平上皮がん，[illegible]，腺がん），乳がん，[illegible]がんなども発生する。

〔結 論〕 P－クロロベンゾトリクロリドの胃内投与により，投与局所の胃がんの他に，肺がん，リンパ系腫瘍等，[illegible]の場合よりも比較的多様な腫瘍発生が認められ，かつ投与量と[illegible]の間には，いわゆる Dose－Response の関係がみられた。[illegible]ベンゾトリクロリドを[illegible]投与した場合とは[illegible]しており，[illegible]の[illegible]が多少異なるとしても，P－クロロベンゾトリクロリドはベンゾトリクロリドと同様の発がん作用を有する可能性がある。

物 質		A	B
塗布量/回		5 μl	
塗布回数/週		2	
塗布期間(週)		30	
頭 数		22	21
期 間(月)		9.3	
皮膚	扁平上皮がん	12	9
	肉 腫	2	1
	乳 頭 腫	2	4
肺 がん		1	3
消化管	食道がん	5	2
	前 胃 がん	2	
	腺 胃 がん	1	
白 血 病		1	
胸 腺 腫		1	2
担腫動物	悪 性	16	12
	良 性	2	3
	合 計	18	15

表1. P－クロロベンゾトリクロリド（A）とベンゾトリクロリド（B）の皮膚塗布発がん

preliminary experiment for skin

写真1. A群の前胃扁平上皮がん (6.2ヵ月後死亡)
squamatous cell cancer

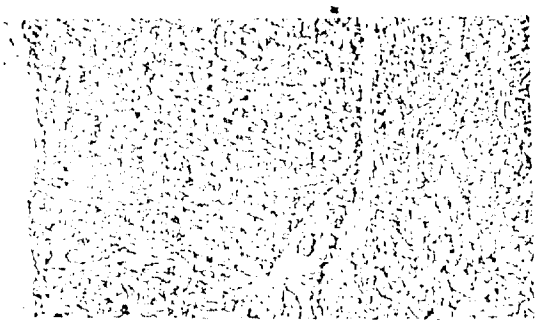


写真2. E群の腺胃腺がん (17.7ヵ月後死亡)
adenocarcinoma of stomach

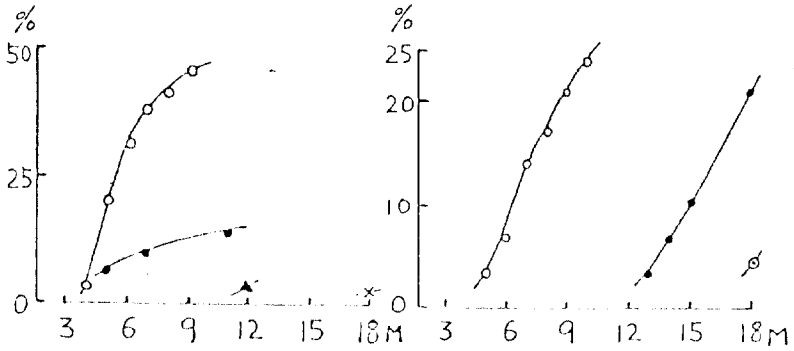


図1 雌ICRマウスの悪性リンパ腫および腺がんの累積発生率
Accumulated rate of lymphoma and adenocarcinoma

図2. 胃がんの累積発生率
Accumulated rate of stomach cancer

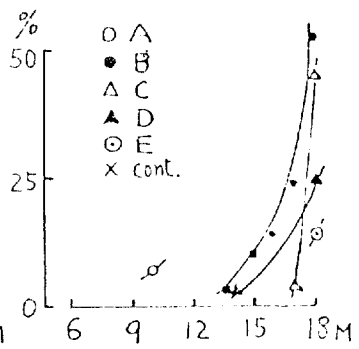


図3. 肺がんの累積発生率
accumulate rate of lung cancer

TABLE 2. TUMORS INDUCED IN ICR MICE FOLLOWING ORAL ADMINISTRATION OF 1-CHLORO-2,4-DINITROBENZENE

GROUP	Initial no. of animals	Effective no. of animals	Mean span of survival (days)	Forestomach: Sq. cell ca.	Sq. cell ca. in situ	Papilloma	Glandular stomach ca.	Lung: Adenocarcinoma	Adenosquamous adenoma	Malignant lymphoma	Thymoma	Skin Tumor	Other Tumors	No. of animals with tumor(s): Malignant	Benign	Total
A 2 μg	31	29	62	7	3	1		2	17	5	8	6[3]	1[6]	16	9	25/29
B 0.7 μg	31	29	168	6	4	2		15	10		4	2[3]	3[3]	20	7	27/29
C 0.2 μg	26	22	169		1	5		10	6			1[4]	1[8]	10	7	17/22
D 0.05 μg	30	28	169			4		7	1	1			1[6]	8	2	10/28
E 0.02 μg	30	27	169			2	1	3	2					4	2	6/22
CONT.	30	26	175						1	1				1	1	2/26

1). Average number in age of effective at the after the treatment. 2). Sq. cell ca. : Squamous cell carcinoma. 3). Sq. cell ca. 4). Sq. cell carcinoma. 5). Sq. cell ca. in situ. 6). Papilloma. 7). One case of oral sq. cell ca. Two cases of salivary gland adenoma were also. 8). Oral sq. cell tumor. 9). Ca. in situ in the stomach was regarded as benign tumor in this case.

TRANSLATION:

CARCINOGENICITY OF P-CHLORO-BENZOTRICHLORIDE *

Fukuda, K.*, S. Matsushita** and K. Takemoto***: *p*-Kuroro-benzotorijurorido no hatsugansei. *Proc. of Japan Assoc. of Ind. Health*, pp. 330-331, 1979.

Purpose of the Study

The raw materials and products handled in the benzoyl chloride production process include toluene, benzotrichloride, benzoyl chloride, benzoic acid, and chlorine gas. Carcinogenicity of benzotrichloride and benzoyl chloride have already been reported at previous sessions of this association's congresses. The reaction by-products and impurities associated with this production process include substances such as benzyl chloride, benzal chloride, *o*- and *p*-chloro-benzo-trichloride, and *o*- and *p*-chlorobenzal-chloride. Although there are variations in the extent of their effects, carcinogenicity of the first four substances has already been recognized by the speakers. Among these, the carcinogenicity of *p*-chlorobenzo-trichloride was found to be comparable to that of benzotri-chloride when applied to the skin surface (Table 1). In the present study, its carcinogenicity, as well as the dose-response relationship, were further investigated via the oral route.

---

*Industrial Medicine Research Institute.

**Industrial Medicine Research Institute, National Public Health Institute.

***Saitama Medical University, Department of Public Health.

Substance		A	B
dosage applied to the skin/application		5 µℓ	
frequency of application/week		2	
duration of application (weeks)		30	
number of animals		22	21
duration (months)		9.3	
skin	squamous cell carcinoma	12	9
	sarcoma	2	1
	papilloma	2	4
lung cancer		1	3
digestive system	esophageal cancer	5	2
	cancer of the forestomach	2	
	cancer of glandular stomach	1	
	leukemia	1	
	thymoma	1	2
tumor-bearing animals	malignant	16	12
	benign	2	3
	total	18	15

Methods

Eight-week-old female JCR-SLC mice were used. Thirty animals were assigned to each group. 0.1 ml of sesame oil containing 2 µℓ (Group A), 0.8 µℓ (Group B), 0.32 µℓ (Group C), 0.13 µℓ (Group D), or 0.05 µℓ (Group E) of *p*-chloro-benzotrichloride was administered to each animal via a stomach tube. The administration was scheduled twice weekly and lasted for 17.5 weeks (35 times over the test period). After 18 months following the initiation of the experiment, the animals were subjected to autopsy for observation of histopathological changes.

Results

The results of the test are summarized in Table 2 and Figures 1, 2 and 3. Table 2 shows those with a higher malignancy among primary tumors of the same organs. Multiple tumors in a single animal are all included in the count. The average age (months) of affected animals includes those of the animals sacrificed at the 18th month. Fifty percent mortality occurred earliest in Group A (4.7 months), followed by Group B (12.3 months), while the mortalities of Groups C [to E] did not reach 50% even at the 18th month. Tumors developed earliest also in Group A (which received the highest dosage). Those developed earliest are malignant lymphoma and thymoma, with their incidences up to the 7th month following the start of administration being 38% (11/29) in Group A and 10% (3/29) in Group B (Figure 1 and Table 2). Cancer of the stomach where the test substance came into direct contact began to develop approximately 5 months after the start of the test. The cumulative incidence of this cancer is less than 25% in each group (the data do not include those of carcinoma *in situ*, Figure 2). With the exception of a single incidence of differentiated tubular adenocarcinoma in Group E, all were epidermoid carcinoma. Incidences of carcinoma *in situ* of the forestomach were noted in the groups which received dosages [over 0.32 μℓ]. Papillomatosis was noted to develop in all the groups (Table 2). Keratinization of the forestomach epithelium was more marked in the groups which received higher dosages. A few examples of atypical epithelia were found in the groups which received lower dosages. Epithelial hyperplasia, atypical adenoid proliferation, and epithelialization of the glandular stomach were noted in a few animals.

Lung cancer showed the highest incidence. Its histological types were limited to adenocarcinoma and adenoma, with a virtual absence of any other histological types. The presence of foci of multiple adenocarcinoma and adenoma

TABLE 2. TUMORS INDUCED IN ICR MICE FOLLOWING ORAL ADMINISTRATION OF *p*-CHLORO-BENZOTRICHLORIDE.

GROUP	Initial No. of animals	Effective No. of animals	Av. mth. in age of animals	Forestomach: Sq. cell ca.	Forestomach: Ca. in situ	Forestomach: Multiple Pap.	Glandular stomach ca.	Lung: Adenoca reinoma	Lung: Multiple adenoma	Malignant Lymphoma	Thymoma	Skin Tumor	Other Tumors	No. of animals with tumor(s): Malignant	Benign	Total
A 2 μl	31	29	6.2	7	3	1	-	2	19	5	8	6[3]	1[6]	16	9	25/29
B 0.8 μl	31	29	14.8	6	4	2		15	10		4	2[5]	3[7]	20	7	27/29
C 0.32 μl	26	22	16.9		1	5		10	6			1[4]	1[8]	10	7	17/22
D 0.13 μl	30	28	16.9			4		7	1	1			1[6]	8	2	10/28
E 0.05 μl	30	22	17.9			2	1	3	2					4	2	6/22
CONT.	30	26	17.5						1	1				1	1	2/26

1) Average months in age of affected animals after the treatment. 2) Sq. cell ca.: Squamous cell carcinoma. 3) Sq. cell ca. 4) Spindle cell carcinoma. 5) Sebaceous gland carcinoma. 6) Mammary adenocarcinoma. 7) One case of ear canal sq. cell ca. Two cases of salivary gland adenocarcinoma. 8) Ovary glanulosa cell tumor. 9) Ca. *in situ* in the stomach was regarded as benign tumor in this case.

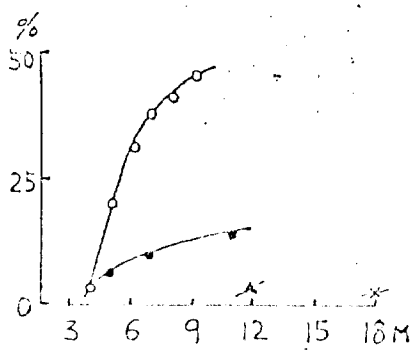


Figure 1. Cumulative incidences of malignant lymphoma and thymoma.

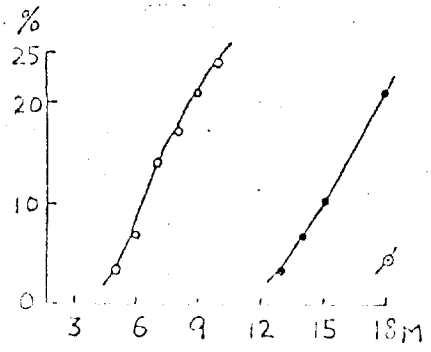


Figure 2. Cumulative incidences of stomach cancer.

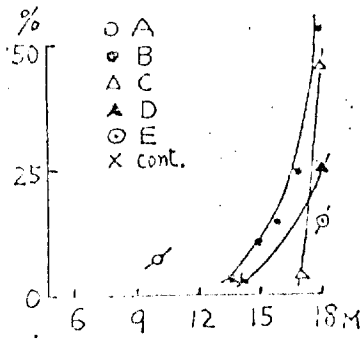
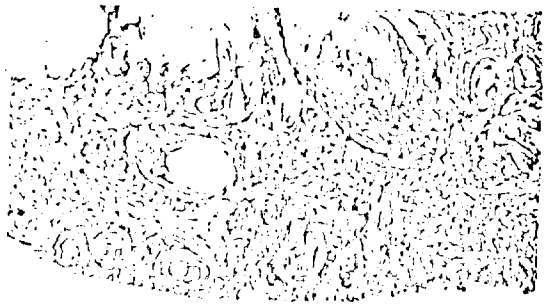
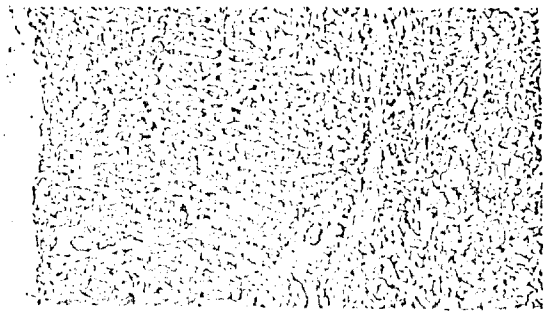


Figure 3. Cumulative incidences of lung cancer.



Photograph 1. Squamous cell carcinoma of the forestomach of Group A (the animal died in 6.2 months).



Photograph 2. Adenocarcinoma of the glandular stomach of Group E (the animal died in 17.7 months).

containing adenocarcinoma at the center was noted. The incidences up to the 18th month were higher in the groups receiving higher concentrations of the test substance (excluding Group A) (Figure 3). Incidences of skin cancer (squamous cell carcinoma, sarcoma, and adenocarcinoma), mammary cancer, and salivary gland cancer were also noted.

Conclusion

Intra-gastric administration of *p*-chloro-benzotrichloride produced -- besides cancer of the stomach where the test chemical comes into direct contact with the tissue -- a relatively wide variety of tumors, such as lymphatic tumors and lung cancer, when compared with direct application to the skin. The dosages and the number of tumor-bearing animals were studied, and a so-called dose-response relationship was recognized. The pattern of tumor incidence was generally similar to that in oral administration of benzotrichloride. The rate of absorption and speed of hydrolysis of the present test agent may be slightly different from those of benzotrichloride, but the carcinogenicity of the two substances is believed to be quite similar.